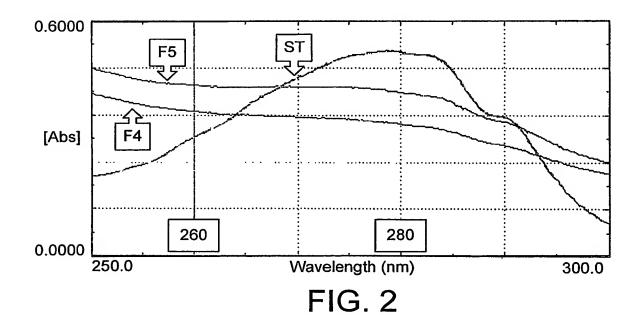


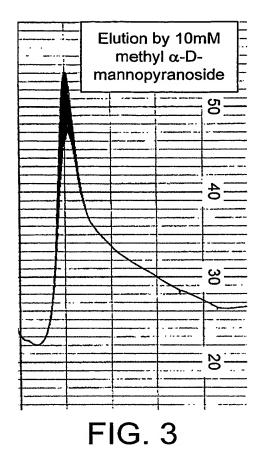
FIG. 1



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0.13500 97.4 A=0.1073 [Abs] 260 280 TUB Nº 7 INSOL. 37°C -0.0150 250.0 Wavelength (nm) 300.0

FIG. 4

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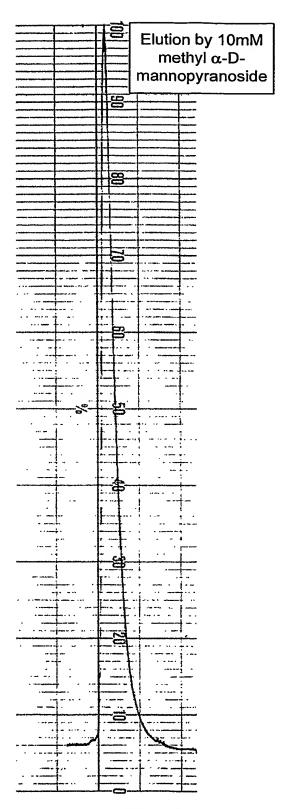
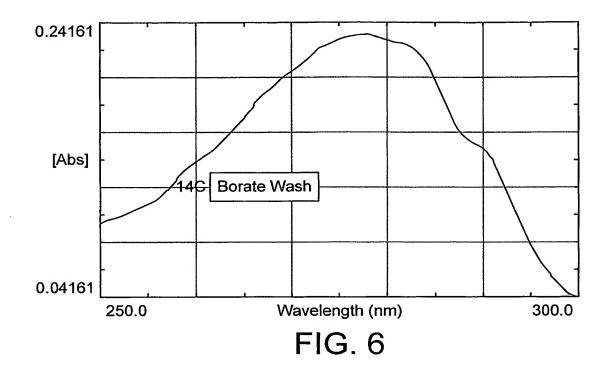


FIG. 5



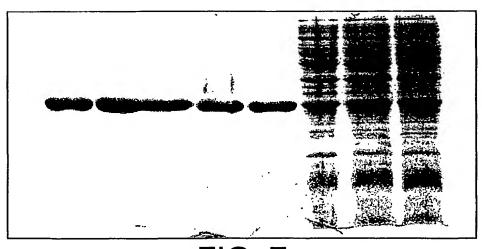
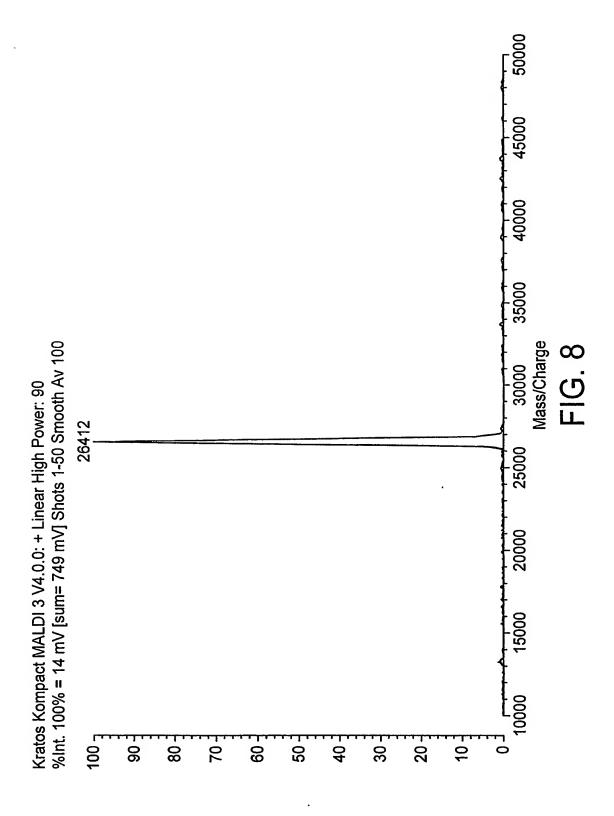


FIG. 7



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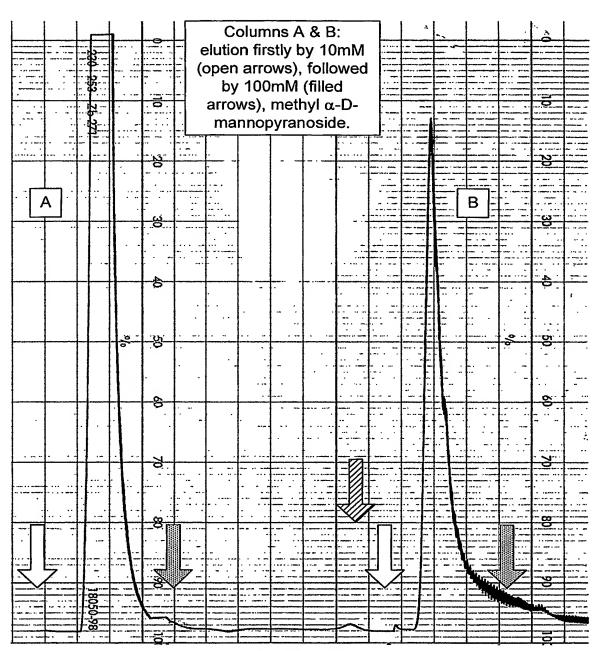
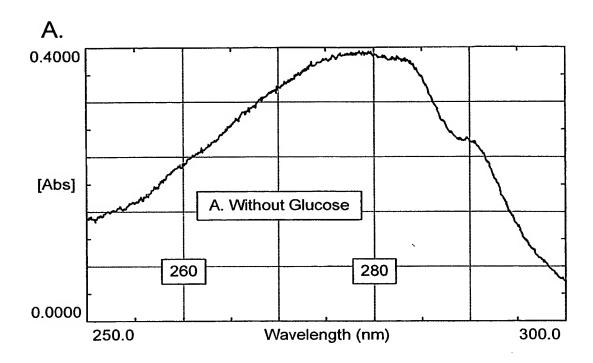
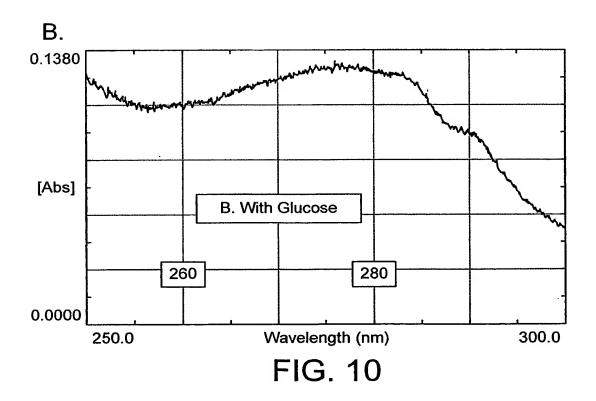
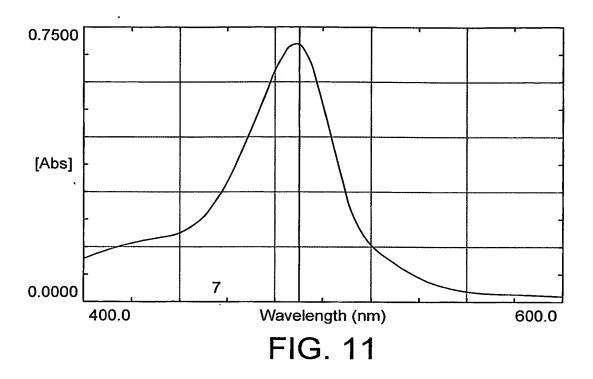


FIG. 9





SUBSTITUTE SHEET (RULE 26)



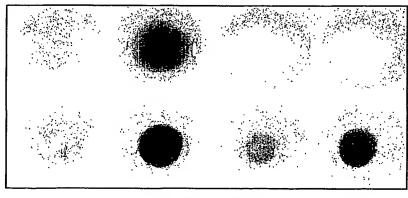


FIG. 12

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SCHEME OF PREFERRED EMBODIMENTS OF METHOD FOR EXAMPLE OF RECOMBINANT FORMS OF CONCANAVALIN A

High-level expression recombinant Con A:

[in glycogen-deficient (mutant) host strain {▶ glycogen eliminated}]

OR

in bacteria grown without glucose {▶ glycogen minimised}



Cell lysis & washing regime (sonication/centrifugation), e.g.:

Borate-EDTA-Triton buffer > Borate-EDTA buffer > water

{recombinant Con A insoluble in pellet – retained,

▶ glycogen released (~borate) + other soluble contaminants – discarded}





Solids dissolved in Guanidine-HCI

{rec. Con A + other proteins denatured – soluble, ▶ residual glycogen, etc. soluble}

Protein refolding regime: buffer dilution, additives, temperature, time

{active rec. Con A + other impurities – soluble,

▶ residual <u>glycogen</u> binds, complex <u>precipitates</u> +
mis-folded proteins aggregate: precipitate centrifuged – <u>discarded</u>}





Solution applied to **Dextran affinity column**

{active rec. Con A binds specifically – retained, inactive Con A, other proteins, etc. wash through – discarded}





Biospecific elution with α-Me-mannopyranoside

{active rec. Con A specifically released - collected}

Analysis for quality and quantity: elution profile, UV-Abs., SDS-PAGE, mass spec., carbohydrate assays

{soluble active rec. Con A – pure, ▶ glycogen & other impurities – absent}

FIG. 13